Polymer(Korea), Vol. 35 No. 5, pp 424-430, 2011

전기분무에 의한 생분해성 폴리포스파젠 마이크로입자의 제조

Li-wei Xue · Qing Cai† · 유승곤* · Ri-guang Jin
Beijing University of Chemical Technology, *충남대학교 화학공학과
(2011년 2월 14일 접수, 2011년 4월 12일 수정, 2011년 4월 12일 채택)

Fabrication of Biodegradable Polyphosphazene Microparticles by Electrohydrodynamic Atomization

Li-wei Xue, Qing Cai†, Seung-Kon Ryu*, and Ri-guang Jin
State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing 100029, China
**Department of Chemical Engineering, Chungnam National University, Daejeon 305-764, Korea*
(Received February 14, 2011; Revised April 12, 2011; Accepted April 12, 2011)

Abstract: Biodegradable poly[(glycine ethyl ester)-(phenylalanine ethyl ester) phosphazene] (PGPP) microparticles were fabricated by electrohydrodynamic atomization to apply drug release test. Atomization parameters such as applied voltage, polymer concentration, and molecular weight were investigated to inspect their effects on the size and morphology of microparticles. The average diameter of PGPP microparticles decreased as increasing applied voltage and solution flow rate. Dichloromethane/ dioxane mixture shows better results for the preparation of microparticles than single solvent owing to the different PGPP solubility in solvent. Blending PGPP polymers with proper molecular weights not only favored the production of spherical PGPP microparticles via electrohydrodynamic atomization, but also provided a way to adjust drug (rifampicin) release behavior. Drug-loaded biodegradable polyphosphazene microspheres can be fabricated via electrohydrodynamic atomization, which has potential use in biomedical applications.

Keywords: polyphosphazene, electrohydrodynamic atomization, microparticle.

Introduction

Electrohydrodynamic atomization (EHDA) technique has been widely employed in fabrication of inorganic nanoparticles,[1] thin films,[2,3] deposition of nanoparticle clusters,[4] micro/nano encapsulation,[5,6] and production of pharmaceutical particles.[7] It becomes a promising method to produce very fine droplets from liquid by using an electrostatic field and by selecting the proper atomization conditions. The droplets can exhibit a narrow size distribution at the micro- or nanometer dimensions. Polycaprolactone (PCL) and poly(lactide-*co*-glycolide) (PLGA) have been mainly employed to prepare drug-loaded microparticles. Recently, exploration of the EHDA to manufacture microparticles for drug delivery is becoming a new focus of research attention.[8-11] Wang *et al.*[12-15] have explored the impact of a number of processing parameters on electrohydrodynamically atomized PCL and PLGA microparticles, and suggested that the properties of solvent and the concentration of polymeric solution were considered the main reasons causing different microparticle diameters and morphologies. Especially concentration gradient resulting from the solvent evaporation in the procedure of EHDA was related closely to the microparticle shapes. However, no other research has been reported on preparation of polyphosphazene microparticles by EHDA.

Polyphosphazene is a relatively new class of polymer, quite distinctly different from all the biodegradable polymers synthesized so far, due to their synthetic flexibility and versatile adaptability for applications. They are linear polymers with inorganic backbones of alternating phosphorous and nitrogen atoms bearing two side groups attached to each phosphorous atom.[16] Because of such unique characteristics of the structure,

†To whom correspondence should be addressed.
E-mail: caiqing@mail.buct.edu.cn

a broad spectrum of biodegradable polyphosphazene can be synthesized by careful tuning the type and ratio of appropriate side groups. The amino acid ester substituted polyphosphazenes are known as a kind of suitable material for the drug sustained release due to their distinctive properties.[17-21] They show fine control of hydrolytic degradation, nontoxic and neutral pH degradation products, and ease of fabrication.[22,23] Preparation of polyphosphazene microparticles by EHDA is thus a significant research subject with great potential in bio-medical applications.

Additionally, different morphologies of microparticles are required so as to meet the needs of varied situations where drug release is applied.[24] Nevertheless, intensive control on the morphology of electrosprayed microparticles had been scarcely concerned in the previous studies, although a number of papers had focused on the electrohydrodynamic atomization of biodegradable aliphatic polyesters.[12-15]

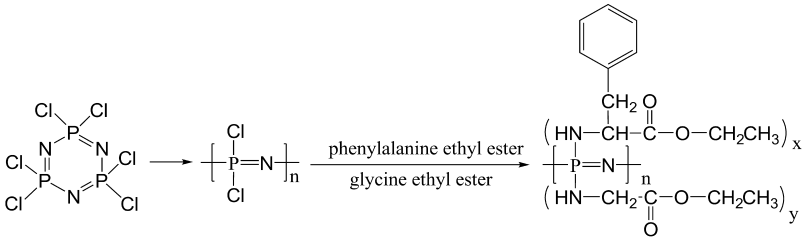
In the light of such a great need, electrohydrodynamic atomization technology was adopted to fabricate biodegradable poly[(glycine ethyl ester)-(phenylalanine ethyl ester) phosphazene] (PGPP) microparticles in the present study. PGPP microparticles with adjustable morphologies and different characteristics were fabricated by changing the atomization parameters such as PGPP concentration in solution, molecular weight of PGPP, mixing ratio of solvents, flow rate and applied voltage. The morphology and size of PGPP microparticles were characterized and related to the atomization parameters.

Experimental

Materials. Hexachlorocyclotriphosphazene (99%, Bo Yuan New Material & Technique Co., China), was recrystallized with anhydrous heptane and vacuum sublimed at 50 ℃ (0.05 mmHg). L-Glycine ethyl ester hydrochloride (99%, Yangzhou Baocheng Bio-Chemical Co., China) and L-phenylalanine ethyl ester hydrochloride (99%, Yangzhou Baocheng Bio-Chemical Co., China) were dried in a vacuum cabinet at 30 ℃ for 12 h before use. Triethylamine and tetrahydrofuran (THF) were refluxed by CaH_2 and sodium respectively, and distilled. Dichloromethane and dioxane were directly utilized without further purification. The other reagents and solvents used were analytical grade (Beijing Chemical Reagent Co., China). Rifampicin (RIF, 99%, purchased from Shenyang Antibiotic C.) was used as the prototype drug to investigate drug release of PGPP microparticles.

PGPP Synthesis. PGPP was synthesized as described in the previous papers,[25-27] and a schematic procedure was presented in Scheme 1. Polydichlorophosphazene ($PNCl_2$, 0.1 mol) prepared by thermal ring-opening polymerization of hexachlorocyclotriphosphazene at 260 ℃ under vacuum condition, was dissolved in 100 mL THF at 25 ℃, and cooled down to 0 ℃. Then it was slowly added to the mixed solution of triethylamine (0.2 mol) and phenylalanine ethyl ester which was prepared by refluxing the mixture of L-phenylalanine ethyl ester hydrochloride (0.6 mol) and triethylamine (0.6 mol) in THF for 3 h. The obtained mixture was vigorously stirred at 0 ℃ for 6 h and at 25 ℃ for 48 h. Subsequently, THF solution of glycine ethyl ester (0.2 mol) (prepared as phenylalanine ethyl ester solution in THF) was added drop-wise into the reaction mixture. The reaction continued at 25 ℃ for another 24 h. After the precipitated triethylamine hydrochloride was removed by filtration, the resulted polymer solution was concentrated by partly vacuum evaporation of THF, followed by precipitated into petroleum ether (bp: 60~90 ℃) to yield a solid polymer. Finally, the polymer was vacuum-dried to constant weight at 30 ℃ and stored in vacuum to avoid exposure to moisture.

Microparticles Fabrication. Microparticles fabrication was performed following the previous papers.[27,28] The electrohydrodynamic atomization apparatus employed in this study was composed of a variable high voltage power supply (voltage range 5~80 kV), a 10 mL vitreous syringe with a blunt stainless steel capillary (inner diameter=0.34 mm), a micro-infusion pump (WZ-50CZ, Zhejiang University Medical Instrument Co., China) and an aluminum plate (diameter, 5 cm) as the collector. The distance between the nozzle and the collector was controlled between 3 and 12 cm. Transparent polymer solutions were prepared by dissolving PGPP in dichloromethane, dioxane or their mixtures and stirred for 48 h at room temperature. Then the solutions were atomized from the vitreous syringe, which was continuously pushed



Scheme 1. Schematic synthesis of poly[(glycine ethyl ester)-(phenylalanine ethyl ester)] phosphazene.

by a micro-infusion pump at a setting flow rate. The positive electrode of high voltage power supply was attached to the tip of the nozzle and the negative electrode to the collector when a fluid jet was ejected. For the preparation of RIF-loaded microparticles, certain amount of RIF powder was added into the PGPP solutions and electrosprayed. Before characterization and further use, all samples were vacuum-dried to remove residual solvent.

In vitro Drug Release. Screw-capped tubes containing 40 mg of RIF-loaded PGPP microparticles and 40 mL of phosphate buffered saline (PBS, 0.10 M, pH 7.4), were incubated at 37±1 ℃ in a thermostat shaker under 100 rpm. At appropriate intervals, the medium was all taken out and measured photometrically at 334 nm (UV-vis spectrophotometer UV-1800, Shimadzu Company, Japan). 40 mL of new PBS was added into the tube and incubation was continued.

Characterization. In general, the intrinsic viscosity and the Martin constant were used to indicate the interaction among polymeric macromolecules as well as the interaction between polymeric chains and solvent. Intrinsic viscosity of the polymer can be calculated following the "one point method".[29]

$$[\eta] = [2(\eta_{sp1} - \ln\eta_r)]^{1/2}/[c] \quad (1)$$

The Martin constant (K_m) can be determined using Martin equation,[30]

$$\eta_{sp2}/[\eta][c] = \exp(K_m[\eta][c]) \quad (2)$$

where, $[\eta]$ represents intrinsic viscosity, η_r represents relative viscosity, η_{sp} represents specific viscosity, and $[c]$ represents the concentration of polymer solution. The Martin equation is well applicable to the viscosity-concentration relationship in dilute and moderately concentrated polymer solutions.[31] Therefore, 1% w/v solution was prepared by dissolving PGPP in dichloromethane, dioxane and their mixture respectively. The intrinsic viscosity ($[\eta]$) and specific viscosities (η_{sp}) were measured at 30 ℃ by using a Ubbelohde viscometer.

The chemical structure of PGPP was analyzed by NMR (AV600, Bruker, Germany, $CDCl_3$, ppm) and Fourier transform infrared (FTIR, Nicolet Nexus-670 IR, Thermotech, GER.) equipments. Field emission scanning electron microscope (FE-SEM, S-4700, Hitachi, Japan) was used to observe microparticle morphologies. Samples were sputter coated with gold (JEOL JFC-1200 fine coater) before being imaged. The average microparticle diameter was measured by counting 120 microparticles from five SEM observations using image analysis software (Image J, National Institutes of Health, USA).

Statistical Analysis. All data presented were expressed as mean±standard deviation (SD). Statistical analysis was performed using ANOVA followed by Bonferroni comparison, and significant levels were considered at p values ≤ 0.05.

Results and Discussion

PGPP Synthesis. Figure 1 and Figure 2 show the ^{1}H NMR and FTIR spectrum of the synthesized PGPP polymer, respectively. The signals in ^{1}H NMR spectrum are assigned in the figure and listed out as follows: 0.8-1.4 ($-CH_2\underline{CH_3}$); 2.9~3.14 ($-NHCH\underline{CH_2}(C_6H_6)-$); 3.74 ($-NHC\underline{H_2}-$); 3.91 ($-NHC\underline{H}CH_2(C_6H_6)-$); 4.14-4.39 ($-C\underline{H_2}CH_3$); and 7.08~7.21 ($-NHCHCH_2(C_6\underline{H_6})-$). The presence of peaks at 1738.54 cm^{-1} (-COO-), 1201.56 cm^{-1} (-P=N-), and 853.66 cm^{-1} (=P-N=) represent the existence of carboxyl group and -P=N- backbone, respectively. All these spectra information confirm the chemical structure of glycine ethyl ester and phenylalanine ethyl ester attaching to the phosphazene backbone. According to the integrating area of peak (f, g, h) and peak (b, c, d), the composition of PGPP was identified

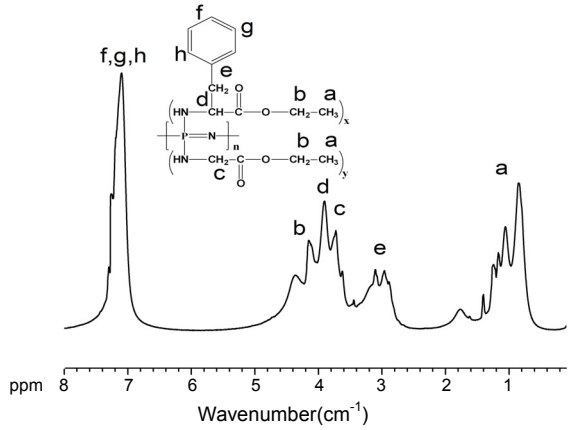


Figure 1. ^{1}H NMR spectrum of poly[(glycine ethyl ester)-(phenylalanine ethyl ester) phosphazene] (PGPP).

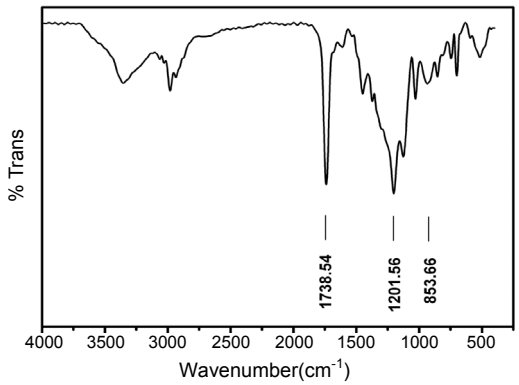


Figure 2. FTIR spectrum of poly[(glycine ethyl ester)-(phenylalanine ethyl ester) phosphazene] (PGPP).

as poly[(glycine ethyl ester)$_{0.33}$ (phenylalanine ethyl ester)$_{0.67}$ phosphazene]. Two PGPP polymers were prepared. Their compositions are similar, but intrinsic viscosities are different. One($PGPP_1$) is 0.58 dL/g(30 ℃, THF) and the other($PGPP_2$) is 0.12 dL/g(30 ℃, THF).

Effect of Solvent on Particle-to-Fiber Formation. Figure 3 shows the effect of solvents on $PGPP_1$ electrohydrodynamic atomization under different polymeric concentrations. As the $PGPP_1$ concentration in dichloromethane increased from 3 to 8% w/v, the atomization products showed morphology change from particles to beaded fibers and further to fine fibers (Figure 3(a-d)). However, particles can be obtained from $PGPP_1$/ dioxane solutions with the concentration as high as 15% w/v (Figure 3(e-f)), but the high boiling point and relative slow evaporation rate of dioxane caused the deformation and adhesion of $PGPP_1$ particles.

As is well known, one of the most important factors to determine whether the polymeric solution would be electrospun into fiber or electrohydrodynamic atomized into particles has been identified as the concentration of the solution.[31,32] It is obvious that the entanglement of macromolecules becomes severe as the solution concentration increasing; in other words, the increase of polymer concentration favors the formation of fibers.[33] This was also confirmed by various forms of electrohydrodynamic atomized $PGPP_1$ products yielded from its dichloromethane solution with different concentrations. Nevertheless, one thing arousing more interest is why particles were resulted from $PGPP_1$/dioxane solutions with high concentration. Probably, different solubility of $PGPP_1$ in the two solvents can interpret this phenomenon.

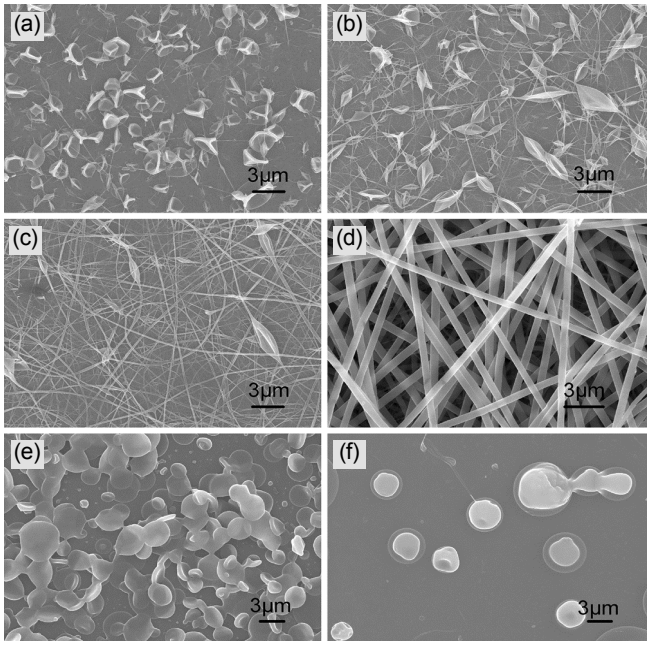


Figure 3. Electrohydrodynamic atomization of PGPP solutions of different solvents and different concentrations: (a) dichloromethane, 3%(w/v); (b) dichloromethane, 4%(w/v); (c) dichloromethane, 6%(w/v); (d) dichloromethane, 8% (w/v); (e) dioxane, 8%(w/v); (f) dioxane, 15%(w/v) (Applied voltage: 20 kV; Flow rate: 0.5 mL/h; Nozzle–plate distance: 6 cm).

The value of intrinsic viscosity represents the fluid dynamic volume of polymeric chains in a selected solvent. As a consequence, if a proper solvent is applied, the macromolecules may have strong interaction with the solvent and extend remarkably, which will result in an increased intrinsic viscosity. If not, the intrinsic viscosity will be decreased. Therefore, it is reasonable to apply the intrinsic viscosity of $PGPP_1$ in dichloromethane or dioxane to compare the solubility. As shown in Table 1, the intrinsic viscosity of $PGPP_1$ in dioxane obtained at 30 ℃ was only 18 mL/g, while it was 108 mL/g in dichloromethane. Apparently, the latter was a better solvent for $PGPP_1$ than the former. As stated above, this would cause the entanglement of polymeric chains to take place at low concentration in the $PGPP_1$/dichloromethane solution. Therefore, fiber structure could be observed even though the $PGPP_1$ concentration in dichloromethane was as low as 3% w/v. The same conclusion could be deduced from the data of Martin constant(K_m) listed in Table 1. The reported studies have proven that larger Martin constant indicated more significant interaction among macromolecules themselves; otherwise, the interaction between polymeric chains and solvent would be prominent.[34,35] The Martin constant of $PGPP_1$ in dichloromethane and dioxane was 0.065 and 1.07, respectively, which clearly demonstrated that the $PGPP_1$ chains were in a shrinking state in dioxane compared to the case in dichloromethane. This feature made molecular overlapping only possible at elevated concentrations in dioxane.

The occurrence of polymeric chain entanglement could be adjusted by changing the mixing ratio of dichloromethane and dioxane. In addition, the presence of fast evaporable dichloromethane helped to solidify the electrosprayed particles to prevent deformation and adhesion. As it can be expected, the intrinsic viscosity of $PGPP_1$ in the mixed solvent decreased and its Martin constant increased orderly with the volume of dioxane increasing (Table 1). Accordingly, the $PGPP_1$ dissolved in mixed solvents with dioxane as the major

Table 1. Intrinsic Viscosity and Martin Constant of PGPP in Dichloromethane, Dioxane and Their Mixtures

Solvent	Mixture of dichloromethane/dioxane (in v/v)				
	1/0	2/1	1/2	1/3	0/1
Intrinsic viscosity (mL/g)[a]	108	81	58	34	18
Martin constant (K_m)[b]	0.15	0.24	1.7	2.83	3.27

[a]Measured by dissolving PGPP in the relative solvent at 30 ℃ with a Ubbelohde viscometer. [b]Calculated from equation (3).

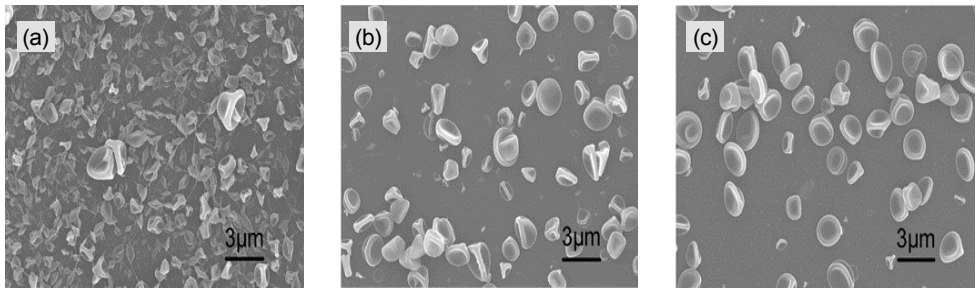


Figure 4. Effect of mixing ratios of dichloromethane to dioxane on the electrohydrodynamic atomization of 8%(w/v) PGPP solution: (a) 2/1(v/v); (b) 1/2(v/v); (c) 1/3(v/v)(Applied voltage: 20 kV; Flow rate: 0.5 mL/h; Nozzle–plate distance: 6 cm).

component, i.e. the volume ratio of dichloromethane to dioxane being 1/2 or 1/3 in this study, could be electrohydrodynamic atomized into dispersed microparticles at the concentration of 8% w/v (Figure 4(b) and (c)) with particle sizes about 1~3 μm. When the mixed ratio of dichloromethane/dioxane was 2/1(v/v), fiber structure could not be avoided at the same $PGPP_1$ concentration (Figure 4(a)). This result suggested that it might be a powerful way to get more desirable $PGPP_1$ microparticles by using a mixture of good and inferior solvent for the electrohydrodynamic atomization of $PGPP_1$.

Effect of PGPP1 Concentration in Mixed Solvent on Particle Morphology. With the mixed dichloromethane/dioxane (1/3, v/v) selected as the solvent, $PGPP_1$ solutions were prepared at four different concentrations. All the solutions were electrohydrodynamic atomized under the same applied parameters. The beads-on-string morphology was clearly observed when $PGPP_1$ was electrohydrodynamic atomized from its 10% wt solution in mixed dichloromethane/dioxane (1/3, v/v), as shown in Figure 5(d). $PGPP_1$ microparticles were obtained from all the other three solutions (Figure 5). The particles turned to be more desirable when the $PGPP_1$ concentration increased.

In the course of electrohydrodynamic atomization, the concentration of the polymeric solution has been noted to have a significant impact on the morphology of microparticles. Under the action of electric force, polymeric solution is drawn from the nozzle, and droplets will form. Then the droplets fall down with solvent evaporation to cause a concentration gradient from the particle surface to the center. Due to the slow migration of polymeric chains toward the droplet center, crusts will be resulted when the surface of the particle solidify. Consequently, if the polymer content is high, small space will be left inside the crust, which will substantially minimize the deformation upon particles. As a result, particles close to spherical shape will be obtained. On the contrary, deformed particles will be resulted due to severe collapse induced by smaller polymer content. Both the photos shown in Figure 3 and Figure 5 can support this theory. As for practical use such as drug carriers, however, it is obvious that more efforts are needed to explore the critical conditions such as molecular weight of $PGPP_1$ and solvent properties for producing $PGPP_1$ microparticles with desired morphology.

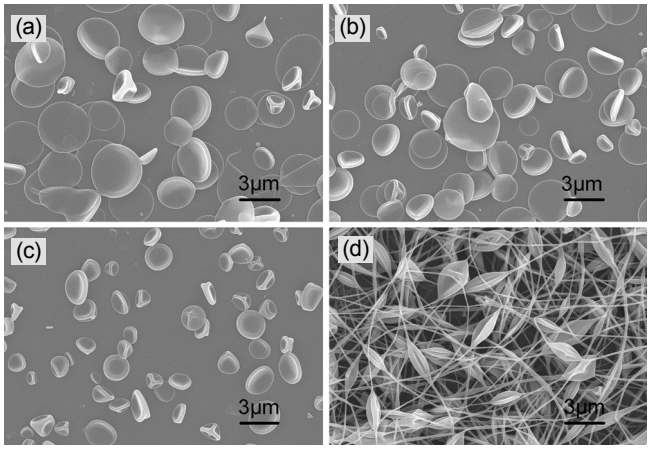


Figure 5. Morphologies of electrohydrodynamic atomizatized PGPP microparticles obtained from PGPP solutions in mixed dichloromethane/dioxane(1/3 v/v) of different concentrations: (a) 4%(w/v); (b) 6%(w/v); (c) 8%(w/v); (d) 10% (w/v)(Applied voltage: 20 kV; Flow rate: 0.5 mL/h; Nozzle–plate distance: 6 cm).

Effect of Molecular Weight of PGPP on the Morphology of Microparticles. As discussed above, to increase the content of $PGPP_1$ in the solution is essential for ameliorating the morphology of electrosprayed microparticles (Figure 5). Unfortunately, it was easy to exceed the critical value to cause fiber formation due to the relatively high molecular weight of $PGPP_1$. And at that stage, the obtained $PGPP_1$ microparticles were far from the spherical morphology, although the application of non-solvent had improved the morphology partly. Blending with polymers of low molecular weights is envisioned as an alternative way to solve this problem. As shown in Figure 6, by blending $PGPP_1$ ($[\eta]_{30\,℃,\,THF}$=0.58 dL/g) with $PGPP_2$ ($[\eta]_{30\,℃,\,THF}$=0.12 dL/g) in the weight ratio of 1:1 or 1:2, fine microspheres were electrosprayed and resulted from the concen-

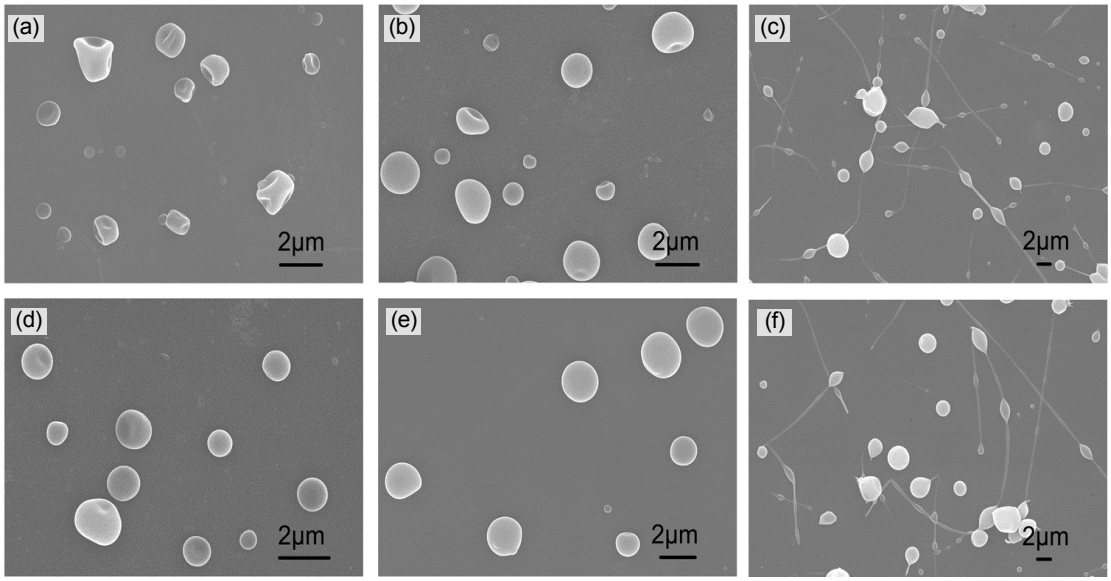


Figure 6. Morphologies of electrohydrodynamic atomized PGPP microparticles obtained from blending PGPP solutions in mixed dichloromethane/dioxane(1/3 v/v) of different concentrations: (a) $PGPP_1/PGPP_2$=1/1, 12%(w/v); (b) $PGPP_1/PGPP_2$=1/1, 14%(w/v); (c) $PGPP_1/PGPP_2$=1/2, 15%(w/v); (d) $PGPP_1/PGPP_2$=1/2, 17%(w/v). (Applied voltage: 20 kV; Flow rate: 0.5 mL/h; Nozzle–plate distance : 6 cm).

trated solutions (12 and 15 wt% in mixed dichloromethane/dioxane (1/3, v/v) solvent, respectively). Fiber structure could be seen at further increased concentrations. Compared to the pure $PGPP_1$ solution, substitution of $PGPP_1$ with a part of $PGPP_2$ had raised the entanglement concentration owing to the smaller excluding volume of $PGPP_2$. The increased proportion of polymer in the electrosprayed droplets, as well as the greater diffusion ability of $PGPP_2$ than $PGPP_1$, both helped to maintain the spherical shape of microparticles after solvent evaporation.

Drug Release of RIF-loaded PGPP Microparticles. RIF-loaded PGPP microparticles were prepared by dispersing the RIF powder in the $PGPP_1/PGPP_2$ blending solutions in the mixed dichloromethane/dioxane (1/3, v/v) solvent. They showed the similar spherical shape to blank ones, and the addition of RIT powder, whose amount was even as high as 10 wt% of the polymer, did not affect the morphology significantly (data not shown). All the PGPP microparticles had the averaged diameter around 1.6 μm with a deviation ~±0.5 μm. Different $PGPP_1/PGPP_2$ blending ratio and varied drug-loading had led to different RIF release behaviors. Apparently, as shown in Figure 7, the release rate increased with the loading content of incorporated RIF increasing gradually, but without significantly initial burst release. And with the percentage of $PGPP_2$ increased, the RIF release was also accelerated when their drug-loadings were at the same level. One interesting thing was that a nearly linear RIF release curve was obtained at the conditions of 3%wt RIF-loading

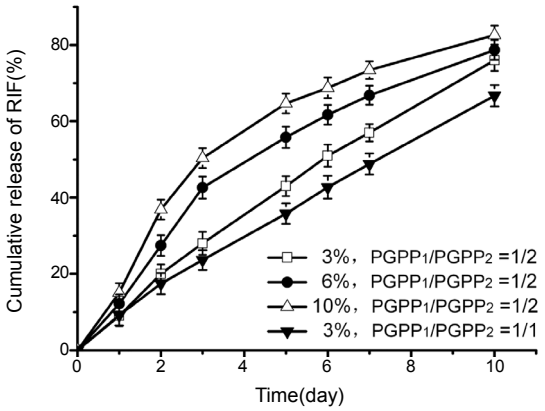


Figure 7. Drug release kinetics of different RIF/PGPP microparticles. The effects of blending ratio of $PGPP_1/PGPP_2$ and drug loading on the cumulative release behavior of RIF.

and weight ratio of $PGPP_1$/ $PGPP_2$ being 1/2. Drug release behaviors were closely related to the diffusion rate of drug molecules in the matrix, which was affected by many factors, including molecular weight, molecular weight distribution and hydrophilicity of matrix, as well as properties and content of drug.[36] That more amount of drug was loaded, and/or more content of polymer with low molecular weight was introduced into the microparticles, contributed to the higher diffusion rate of RIF molecules in $PGPP_1/PGPP_2$ blending microparticles, thus resulted in faster release.

Conclusions

Biodegradable PGPP microparticles can be successfully

and easily produced by electrohydrodynamic atomization. It has been found that mixed solvents from good and inferior solvents demonstrated better results in PGPP electrohydrodynamic atomization compared with single solvent and is an effective way to produce particles or fibers by adjusting the polymeric chain entanglement in solution state. The microparticle morphology was also found to depend significantly on the concentration of polymeric solution, molecular weight of polymer and solvent evaporation. Blending PGPP polymers with different molecular weights not only favored the production of spherical PGPP microparticles via electrohydrodynamic atomization, but also provided a way to adjust the drug release behavior. The electrosprayed biodegradable polyphosphazene microparticles could be prospective carriers for various drugs and find applications in many biomedical fields for the unique properties of polyphosphazenes.

Acknowledgment: The authors acknowledged the financial support from National Natural Science of China (Grant No. 50873012 and No. 51073016).

References

1. I. W. Lenggoro, B. Xia, and K. Okuyama, *Langmuir*, **18**, 4584 (2002).
2. G. J. Buchko, L. C. Chen, Y. Shen, and D. C. Martin, *Polymer*, **40**, 7397 (1999).
3. I. Uematsu, H. Matsumoto, M. Kenji, M. Minagawa, A. Tanioka, Y. Yamagata, and K. Inoue, *J. Colloid Interf. Sci.*, **269**, 336 (2004).
4. S. N. Jayasinghe, M. J. Edirisighe, and D. Z. Zhang, *Nanotechnology*, **15**, 1519 (2004).
5. C. Berkland, D. W. Pack, and K. Kim, *Biomaterials*, **25**, 5649 (2004).
6. I. G. Loscertales, A. Barrero, I. Guerrero, R. Cortijo, M. Marquez, and A. M. Ganan-Calvo, *Science*, **295**, 1695 (2002).
7. J. C. Ijsebaert, K. B. Geerse, J. C. M. Marijnissen, J. J. Lammers, and P. Zanen, *J. Appl. Physiol.*, **91**, 2735 (2001).
8. B. Bugarski, Q. Li, M. F. A. Goosen, D. Poncelet, R. J. Neufeld, and G. Vunjak, *AIChE J.*, **40**, 1026 (1994).
9. T. Ciach, *Int. J. Pharm.*, **324**, 51 (2006).
10. K. B. Geerse and J. C. M. Marijnissen, *Electrospray as Means to Produce Monodisperse Drug Particles*, L. Grado'n and J. C. M. Marijnissen, Editors, Kluwer Academic Publishers, The Netherlands, pp 75-90 (2003).
11. S. H. Hong, J. H. Moon, J. M. Lim, S. H. Kim, and S. M. Yang, *Langmuir*, **21**, 10416 (2005).
12. D. Luna, L. Timothy, and W. Chi-Hwa, *J. Control. Release*, **102**, 395 (2005).
13. X. Jingwei, J. C. M. Marijnissen, and W. Chi-Hwa, *Biomaterials*, **27**, 3321 (2006).
14. X. Jingwei, L. Liangkuang, P. Yiyong, H. Jinsong, and W. Chi-Hwa, *J. Colloid Interf. Sci.*, **302**, 103 (2006).
15. Y. Jun, L. L. Kuang, X. W. Jing, H. J. Song, and W. Chi-Hwa, *J. Aerosol Sci.*, **39**, 987 (2008).
16. H. R. Allcock, *Chemistry and Applications of Polyphosphazenes*, Wiley- Interscience, Hoboken, NJ., 2003.
17. S. Lakshmi, D. S. Katti, and C. T. Laurencin, *Adv. Drug Deliver. Rev.*, **55**, 467 (2003).
18. C. T. Laurencin, H. J. Koh, T. X. Neenan, H. R. Allcock, and R. Langer, *J. Biomed. Mater. Res.*, **21**, 1231 (1987).
19. H. R. Allcock, S. R. Pucher, and A. G. Scepelianos, *Biomaterials*, **15**, 563 (1994).
20. F. M. Veronese, F. Marsilio, P. Caliceti, P. De Filippis, P. Giunchedi, and S. Lora, *J. Control. Release*, **52**, 227 (1998).
21. F. M. Veronese, F. Marsilio, S. Lora, P. Caliceti, P. Passi, and P. Orsolini, *Biomaterials*, **20**, 91 (1999).
22. H. R. Allcock, T. J. Fuller, D. P. Mack, K. Matsumura, and K. M. Smeltz, *Macromolecules*, **10**, 824 (1977).
23. H. R. Allcock, S. R. Pucher, and A. G. Scopelianos, *Macromolecules*, **27**, 1071 (1994).
24. D. Nishit, P. Balabhaskar, R. R. Angela, P. Kapil, S. Shivshankar, and M. Samir, *J. Control. Release*, **146**, 196 (2010).
25. H. R. Allcock, T. J. Fuller, D. P. Mack, and K. Matsumura, *Macromolecules*, **10**, 824 (1977).
26. H. R. Allcock, W. J. Cook, and D. P. Mack, *Inorg. Chem.*, **11**, 2584 (1972).
27. T. Zhang and Q. Cai, *Polym. Mat. Sci. Eng.*, **22**, 90 (2006).
28. Q. F. Qin, Y. Q. Dai, K. Yi, L. Zhang, S. K. Ryu, and R. G. Jin, *Carbon Letters*, **11**, 176 (2010).
29. R. S. Cheng, *Polym. Commun.*, **4**, 159 (1960).
30. J. T. Yang, in *Advances in Protein Chemistry*, Academic Press, New York, Vol **14**, p 323 (1961).
31. H. Fong, I. Chun, and D. H. Reneker, *Polymer*, **40**, 4585 (1999).
32. K. H. Lee, H. Y. Kim, H. J. Bang, Y. H. Jung, and S. G. Lee, *Polymer*, **44**, 4029 (2003).
33. M. G. McKee, G. L. Wilkes, R. H. Colby, and T. E. Long, *Macromolecules*, **37**, 1760 (2004).
34. W. K. Son and J. H. Youk, *Polymer*, **45**, 2959 (2004).
35. M. R. Kasaai and G. Charlet, *Food Res. Int.*, **33**, 63 (2000).
36. H. Youliang, L. Yanyan, Y. Yizi, L. Dongmei, and Z. Guangtian, *J. Aerosol Sci.*, **39**, 525 (2008).